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Diplodia Infection of Hevea Buddings and Its Prevention by the Use of a Protective Fungicide[1]

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INTRODUCTION

The practice of budding, also called patch budding and bud grafting, is of utmost importance in the commercial propagation of the Para rubbertree, *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg. Clones giving high yields of rubber have been selected (*1*, *4*, *11*),[3] and base budding of seedling rootstocks with these clones is used to pass on this very desirable high-yielding characteristic.

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[2] Grateful acknowledgment is made to Ingenieros Manuel Puente Cerda, director, and Angel Vergara Castillo, agronomist, Campo Experimental de Hule, for their valuable assistance and suggestions in this research.

[3] Italic numbers in parentheses refer to Literature Cited, p. 22.

In the Western Hemisphere there is a severe leaf disease of hevea called South American leaf blight, caused by *Dothidella ulei* P. Henn., which formerly made it impossible to have successful commercial plantations. Clones resistant to this disease have been selected under a cooperative program of rubber investigation with Latin American countries (*9*), but, unfortunately, most of them are of very low yield. Thus, for successful commercial production in this hemisphere, it is still necessary first to low-bud seedling rootstocks, at a height of 1 to 2 inches, with a clone of high production and then later to top-bud this clone, at 6 to 7 feet, with a resistant clone.

Budding of hevea will continue to be of importance until a source of seed is available that will produce trees that are both resistant and high yielding. This is a very difficult thing to do: and, because of the time-consuming nature of research in this field, it is unlikely that the problem of obtaining such seed will be resolved in the near future.

Top-budding results in Mexico[4] in past years have invariably been unsatisfactory. Seldom has it been possible to obtain as much as 25 percent success, and frequently the successful top buddings were less than 5 percent of the total number of buddings made. The base-budding results obtained were, in general, much better, but even they were at times unexplainably low. Numerous investigations have been made by various workers in an attempt to improve budding success, with only indifferent results. The factors investigated included length of time buddings should be left wrapped; stage of development of the terminal bud of the budwood and shoot; effect of girdling of the budwood some weeks prior to its use; effect of rainfall, temperature, and humidity; effect of variations in budding technique; differences between clones; and effect of the application of various hormones and of lanolin (*3*) and vaseline.

In the course of the present investigation, infection of the bud patch by the fungus *Diplodia theobromae* (Pat.) Nowell was found to be a major cause of budding failure. *Diplodia* is commonly reported as a wound parasite, and, since the separation of the bud patch from the budwood represents almost the ultimate in wounding, the infection of the bud patch by *Diplodia* or other wound parasites is not to be wondered at.

REVIEW OF LITERATURE

There has been no previous report, so far as is known, of a specific fungus being held responsible for failure of hevea buddings. Summers (*11, p. 70*), in discussing the length of time a budding should be left wrapped, gives the following general statement on the possibility of attack of the bud patch by fungi or bacteria:

> The budding is left in the stage described above (i. e., wrapped) for a period of 14 days. Opening up may then be begun but, in any case, it should not be delayed longer than the 21st day as every extra day increases the risk of moisture penetrating the binding strip and causing the bud-patch to rot through the attack of fungi and bacteria.

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[4] VERGARA CASTILLO, A. INVESTIGACIONES SOBRE EL INJERTO DEL HEVEA. [Unpublished thesis. Copy on file Esc. Nac. Agr., Chapingo, Mexico.]

Summers, however, does not back his statement up with experimental evidence.

A disease caused by *Chalaropsis thielavioides* Peyronel has been reported by Hamond (*2*) as affecting grafts of nursery walnut trees (*Juglans nigra* L.). The grafts were made on 1-year-old trees at about 3 inches above the ground. A double whip-and-tongue graft was used. The disease caused an almost complete failure of the walnut grafts, because it killed the stock and scion tissues at the point of union. The causal organism was isolated in pure culture, and positive results obtained from inoculation gave definite evidence that the fungus was the cause of the disease. A solution of 1 part of 40-percent formaldehyde to 39 parts of water was used to control the fungus. This solution was sprayed around in the greenhouse, where the grafting was done, and it was also applied to the walnut stock before it was cut at the point of union at which the graft was to be made.

A disease of rose grafts, also caused by *Chalaropsis thielavioides*, has been reported by Longrée (*7*). The disease prevented the formation of callus and the "taking" of grafts and caused the death of the scion involved. The cut surfaces of the grafts became covered with the mycelium and spores of the fungus, whose pathogenicity on rose was demonstrated. The fungus at times also penetrated deeply into the tissue of both stocks and scions. All reported cases of the disease could be traced back to shipments of stocks from a specific area, which indicates that the pathogen was carried on the stock plants.

There have been numerous reports (*6*, *8*, *10*, *13*) on diseases of hevea and other tropical plants, especially cacao, caused by or associated with *Diplodia*. This fungus has been reported from almost every country in the Tropics. It occurs commonly as a saprophyte on a wide variety of dead or dying plant material and can maintain itself indefinitely under tropical conditions by a saprophytic existence.

Considerable confusion exists regarding the taxonomy of the genus *Diplodia*. According to Voorhees (*12*), *Diplodia theobromae* should be included as a synonym under *Physalospora rhodina* (Berk. & Curt.) Cke. However, since neither this nor any other perfect stage has been definitely proved to belong to the *Diplodia* occurring on *Theobroma* or *Hevea*, it is considered best to retain the use of the name of the imperfect stage, as is now permitted under the International Rules of Botanical Nomenclature.

Sharples (*10*) gives an extensive account of the conditions under which *Diplodia* appears to be parasitic on hevea. "Diplodia dieback" commonly affects trees that are below normal in vigor. *Diplodia* also rapidly follows any damage done by scorching. The fungus is always prominent in the following affections: Lightning damage on both old and young trees, sun-scorch of exposed lateral roots, seedlings affected by excessive ground heat at the collar, dieback in large snags after the budding operation is done, and spearhead wounds at junction of scion and stock.

La Rue and Bartlett (*6*) made extensive isolations and inoculations with *Diplodia* on hevea, especially on moldy rot of the tapping panel. In the summary of their results, they stated (*pp. 106–107*):

> It is fair to conclude that much of what is called "black thread" disease is due to Diplodia, and that the fungus primarily concerned in moldy bark is likewise Diplodia.

MATERIALS AND METHODS

The major part of the work reported in this circular was carried out from 1948 to 1951 at the Campo Experimental de Hule, El Palmar, Veracruz, Mexico, in cooperation with the Mexican Ministry of Agriculture.

Isolations of *Diplodia theobromae* were made from dead bud patches of unsuccessful buddings and from infected bark surrounding the budding. The material was surface-sterilized in 1:1,000 mercuric chloride in 65-percent alcohol for 1 minute and washed in sterile tap water. It was then sectioned aseptically, and the pieces were placed in petri dishes on potato-dextrose agar. Transfers to test tubes were made from the borders of colonies growing from the pieces. Some of these transfers were used later in inoculation experiments with bud patches and buddings.

Inoculation experiments with *Diplodia* and other fungi were performed on bud patches in the laboratory and on buddings in the field. The bud patches for the laboratory inoculation trials were cut from green-barked budwood in the regular manner and also with aseptic precautions. They were then placed in previously sterilized moist chambers consisting of two glass slides on moist filter paper in a petri dish. One bud patch was placed on each slide and inoculation was accomplished by placing some of the inoculum in contact with the bottom end of the patch. Buddings in the field were inoculated either by placing inoculum in contact with the base of the budding or by dusting with spores after the bud patch was in place.

In testing the effects of fungicides on *Diplodia*, a glass-ring petri-dish method was used. Potato-dextrose agar was poured into petri dishes and sterile glass rings (¼ inch high and ½ inch in diameter), three or four to a plate, were placed in the agar before it solidified. A basal concentration, in water, of the fungicide to be tested along with two progressive dilutions of a 1:10 ratio made from it were used. Then ¼ cc. of the fungicide was pipetted into a ring in each of three dishes, giving three replicates for each concentration of the fungicide. After the fungicide had been added to the rings, the dishes were inoculated by dusting liberally with *Diplodia* spores. In 5 days, data were taken on the effect of the various fungicides on fungus growth. Fungus growth within the ring was rated as H, heavy; M, medium; L, light; and N, none. If inhibition extended beyond the ring, it was recorded in millimeters of inhibition beyond the ring.

Tests were also made of the toxicity of the fungicides to the cambium of hevea. Branches of pollarded trees of various panel clones, 1 inch to 1½ inches in diameter, were used. The bark was cut to simulate top-budding procedure, using a flap about 3 inches long, which was opened at the bottom. After the latex was dry, the flap was lifted and the fungicide was dusted or sprayed onto the exposed cambium. The flap was then put back into place and wrapped with a new tape. Three replicates of each treatment were made. Notes on injury to the cambium and to the flap were taken in 6 days.

In the top-budding experiments using the various fungicides, the standard method of budding was followed and, generally, the flapless type of budding was employed. The standard procedure for budding hevea has been extensively treated elsewhere (*1, 4, 11*); and, therefore, it will only be reviewed here. Fundamentally, the procedure consists of removing a patch of bark, approximately ½ inch wide by 2 inches long, from the stock plant and putting in its place a similar-sized patch, containing a bud in its center, from the clone that is to be multiplied. Special care must be taken that latex does not get under the transplanted patch, as the resultant film of rubber between the cambial layers of the patch and the stock would act as an effective barrier to prevent their union.

The buddings are treated with a fungicide as follows: The fungicide is mixed with water to get the desired concentration. Before the bud patches are cut, the stick of budwood is wiped with a cloth moistened with the fungicidal preparation and is allowed to dry. This leaves a film of fungicide on the bark. The budding panels on the stock plants are cut in the usual manner, and the exuded latex is allowed to coagulate. Then the coagulated latex is wiped off with the fungicide-moistened cloth; this leaves a protective coating of the fungicide around the marked-out panel. The usual budding technique is then followed; i. e., the flap is opened or pulled off, the bud patch is inserted, and the budding is wrapped. The fungicide was not applied to the exposed cambium of bud patch or shoot. Twenty buddings were made for each replicate in a treatment, and they were left wrapped for 15 to 30 days.

THE CAUSAL ORGANISM

Isolations From Unsuccessful Buddings

Numerous isolations were made of the *Diplodia* fungus, especially in connection with budding failure. When the isolations from unsuccessful buddings were made from recently killed bud patches or from the borders of the infected parts, *D. theobromae* was generally the only fungus present. *Diplodia* was found in 104 out of 105 portions of infected material plated out in one series of isolations made from bud patches, flaps, and surrounding bark of 5 unsuccessful buddings. Only 3 of the portions had 1 sector each of other fungi. In another series of isolations, also made from 5 unsuccessful buddings, 90 portions were found to contain *Diplodia*, and no other fungus other than a *Penicillium* sp. was present in 55 portions.

Characteristics of the Fungus

Pycnidial production in pure culture was very sparse and, especially in test tubes, was often obscured by a thick mass of dark-colored mycelium. This was true of the fungus grown on potato-dextrose agar, on potato agar, on malt-dextrose agar, and on twigs of hevea in water agar. The pycnidia were produced only after the cultures had begun to dry.

Pycnidia were produced abundantly on hevea budwood and were large enough to protrude from the bark and could be seen with the naked eye (fig. 1). The spores are expelled while still one-celled and hyaline (fig. 2, *A*) in the form of long (approximately ⅛ inch), white, curling threads, or cirri, under conditions of high humidity (fig. 3, *A*). If the high humidity persists for about 36 hours, the spores change to

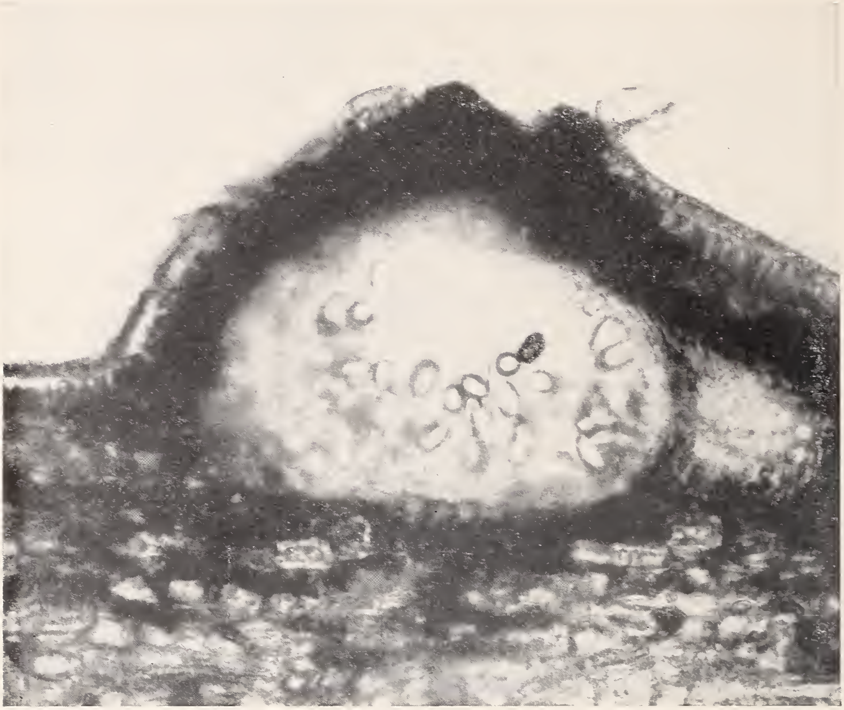

FIGURE 1.—Cross-section of a pycnidium of *Diplodia theobromae* protruding from the bark of a young branch of hevea. Note the hyaline, one-celled condition of the spores within the pycnidium. × 125.

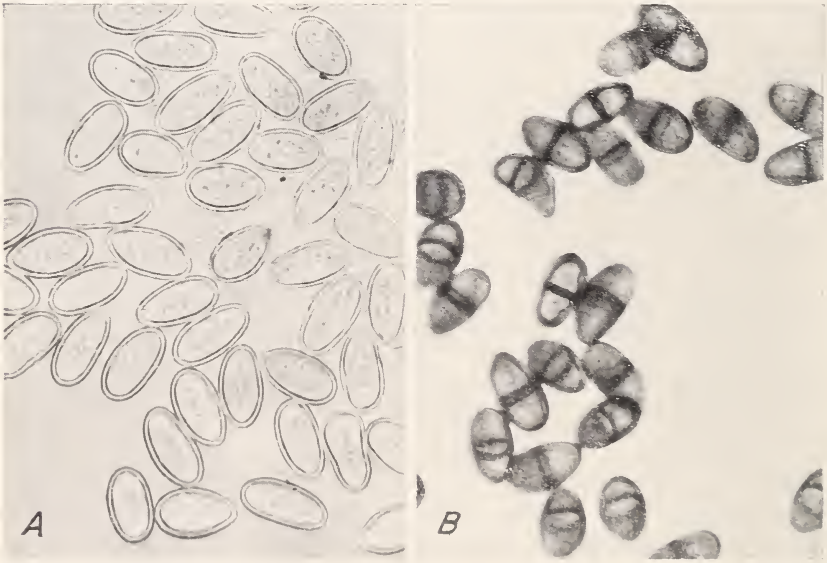

FIGURE 2.—Spores of *Diplodia theobromae*: *A*, Immature, hyaline, one-celled stage, as expelled from the pycnidium; *B*, same spores in mature, dark-colored, two-celled stage. × 400.

the two-celled, dark-colored stage (fig. 2, *B*) characteristic of *Diplodia*, covering the surface of the bark as a dark, powdery mass (fig. 3, *B*). However, the spores will remain in the one-celled hyaline stage indefinitely under conditions of low humidity. If the surface and atmosphere are sufficiently moist, the spores will germinate in place and cover the surface with a profuse growth of dark-colored mycelium.

Checks of spore measurements gave essentially the same limits of 12μ to 15μ by 25μ to 30μ, as given by Nowell (*8*).

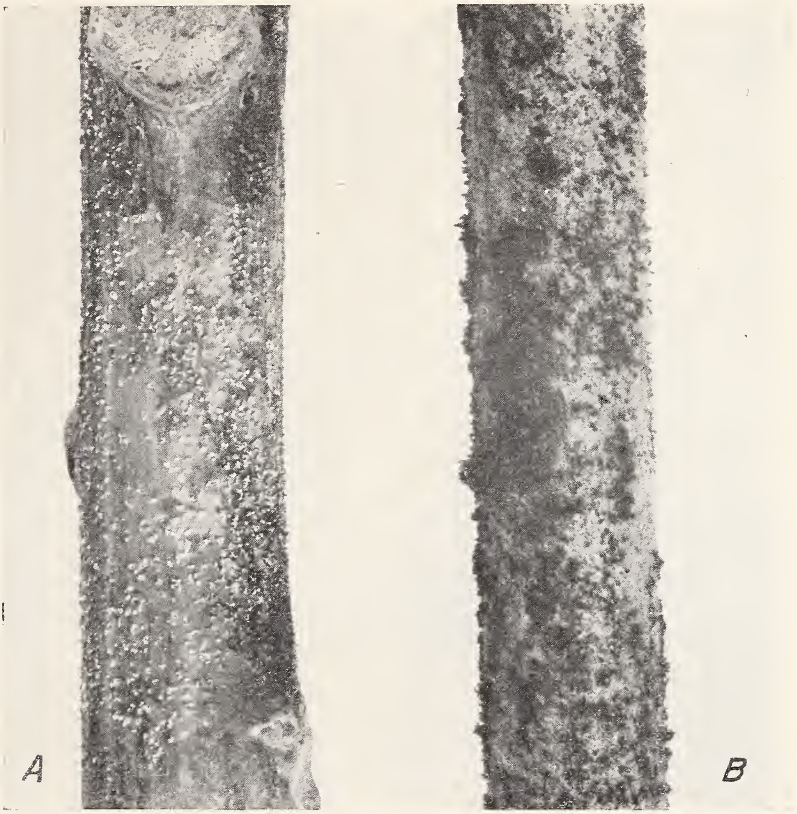

FIGURE 3.—Macroscopic appearance of spores of *Diplodia theobromae* on the surface of young hevea branches: *A*, In white, curling cirri, as first expelled from the pycnidia; *B*, black, powdery masses of spores on maturing, approximately 36 hours later. × 3.

The possibility that the *Diplodia theobromae* attacking cacao pods and the one attacking hevea branches are of different strains was indicated by the results of a preliminary cross-inoculation test. Wounding and subsequent inoculation of green cacao pods with the *Diplodia* from hevea gave negative results, as did the inoculation of hevea budwood with *Diplodia* from cacao.

OCCURRENCE OF *Diplodia theobromae*

In Mexico *Diplodia theobromae* has been found on the leaves, branches, and maturing seed pods of hevea, either alone or in conjunction with other fungi. On leaves it is occasionally present in lesions caused by other fungi. It is very commonly encountered in connection with dieback of the branches of trees weakened by leaf blight.

Sunscald, which occurred frequently on trees that had been pollarded, was invariably followed by *Diplodia*. In fact, the first indication of sunscald injury on the trunks was the grayish-black discoloration caused by fructifications of the fungus.

In general, *Diplodia* enters and advances rapidly in any branch of the tree that is in a weakened condition. The inner and lower branches that are dying back in the course of natural pruning may be attacked as well as the stub remaining above the bud patch after the stock plant has been cut back and the upper part of a three-component tree transplanted just prior to an unfavorably dry spell. *Diplodia* can also quickly invade live branches pruned or broken from the tree.

Extensive dieback of the top-budded cut-back branches of pollarded trees occurred during the dry season before the buddings were able to shoot, and especially if there were other branches that had not been budded and cut back on the same tree. *Diplodia* was invariably present in the dying stubs and probably was an important factor in hastening their death.

If a short flap, the top of which does not recontact the cambium above the bud patch, is used, the flap will very commonly be invaded by *Diplodia*, which often spreads to the bud patch. Bud patches of unsuccessful buddings will generally contain pycnidia and spores of *Diplodia*. Often, especially if moisture has penetrated the wrapping, *Fusarium* sp., *Phyllosticta* sp., and *Gloeosporium* sp. also will be found. In an inspection of 150 unsuccessful buddings made on unwrapping 30 days after budding, 54 had easily visible, sporulating fructifications of *Diplodia*.

Pycnidia of *Diplodia* also were found in the waxed-cloth wrapping tapes that had been used for budding. Probably most of the discoloration of used tapes is the result of growth of mycelium of the fungus within them. The possible danger from the re-use of such fungus-infested tapes for budding purposes is obvious.

A microscopical examination of 10 dead bud patches of top buddings made at Turrialba, Costa Rica, showed that 4 were infested with *Diplodia theobromae*, 2 with *Gloeosporium* sp., 1 with both *Gloeosporium* sp. and *Phyllosticta* sp., and 3 had no spore forms. The fungus present in dying-back stubs above the budding on three-component trees was *Gloeosporium* sp. or *Phyllosticta* sp. rather than *Diplodia*, as in Mexico. This may be because of the lower prevailing temperature in Costa Rica, especially at Turrialba. Preliminary evidence indicates that the *Gloeosporium* sp. will advance faster than *Diplodia* in hevea budwood during cooler weather.

INOCULATION OF BUD PATCHES AND TOP BUDDINGS WITH *DIPLODIA THEOBROMAE*

Inoculation of Bud Patches in the Laboratory

The bud-patch inoculation tests were made in January 1949. Bud patches were taken from clone FB 3363, one of the standard leaf-blight-resistant top-budding clones. Three treatments were used:

(1) Control; (2) budwood, hands, and knife washed with 70-percent alcohol; and (3) as in (2) but buddings afterward inoculated by placing a small piece of dead bud patch from an unsuccessful budding, containing pycnidia of *Diplodia theobromae*, in contact with the bottom of the bud patch. The bud patches were placed in petri-dish moist chambers. Ten bud patches were used in each treatment.

The results after 10 days were as follows: (1) In the control series, 8 patches were alive, 2 dead; (2) in the aseptic series, 10 patches were alive; and (3) in the inoculated series, 10 patches were dead. Pycnidia of *Diplodia* appeared on all the inoculated patches within 15 days. The bud patches of the aseptic series were still alive and green after 15 days. Most of these patches had ordinary saprophytic fungi growing around their cut edges, as evidenced by visible mycelium. The results clearly demonstrated that there was a fungus, probably *D. theobromae*, in the dead bud patches that actively parasitized the fresh bud patches.

Similar results were obtained when an isolate of *Diplodia* was used for inoculation; its pathogenicity for hevea bud patches was thus demonstrated. An isolate of *Colletotrichum* obtained from shoots of hevea afflicted with dieback also caused death of bud patches, but it was slower in its action than *Diplodia.*

Inoculation of Top Buddings in the Field

A preliminary top-budding trial was made in January 1949, an unfavorable month for budding because of lack of rainfall. An aseptic budding technique was used in an attempt to exclude a possible fungal factor. Flapless buddings were made, and Scotch tape was used for wrapping to provide easy visibility. Green budwood of clone FB 3363 was selected, since infection is more easily observed in green than in brown budwood. There were two series of buddings. In one treatment, used as a control, no precautions were used. In the other treatment, the hands of operators, the knife used in budding, and the shoots and budwood were washed with 70-percent alcohol. Eleven buddings were made for each series.

The results of the experiment, as determined when the buddings were unwrapped after a period of 10 days, were as follows: In the control series, 18 percent of the buddings were alive, nine being dead and the upper half of the patch being alive in two. In the treatment in which the aseptic technique was used, 64 percent of the buddings were alive, four being dead, four being alive in the upper half, and three being uninfected. Thus, although the fungus was apparently able to infect the buddings, the aseptic technique gave partial control of infection.

A field-scale top-budding inoculation experiment was made in April, well in advance of the most favorable season for budding. Three treatments were used in this experiment. One series of buddings was made without regard to infection. In the second series of buddings, the base of the bud patch was inoculated with a small quantity of mycelium taken from an agar culture of *Diplodia;* and in the third series Fermate was applied to the shoot and budwood.

The buddings were left wrapped for 20 days, and they were rated (table 1) 20 days after unwrapping. Each figure in the table represents 20 buddings. A completely healthy budding was given a rating of 5; a lightly infected budding, 4; and a budding with the lower half of the bud patch dead but not including the bud, 2; and the remainder were rated as 0.

The ratings show conclusively that infection by *D. theobromae* can be a major factor in limiting budding success. Inoculated buddings received an average rating of 26.0 as compared with 71.3 for untreated control buddings. When Fermate treatment was given, budding success was increased to 100.

In a smaller inoculation trial on essentially similar lines as those followed in the experiment outlined, inoculation with *Colletotrichum* lowered the budding success to practically the same degree as inoculation with *Diplodia.*

TABLE 1.—*Results of inoculation of buddings with Diplodia theobromae by placing a small quantity of inoculum in contact with the base of the budding* [1]

Treatment	Bud-patch-infection results			
	By budder No.—			Average
	1	2	3	
	Rating	*Rating*	*Rating*	*Rating*
Control	71	75	68	71. 3
Inoculated	34	30	14	26. 0
Fermate-treated	100 100 100	100 100 100	100 100 95	100. 0 100. 0 98. 3

[1] Each rating represents a cumulative rating for 20 bud patches on the basis of 5 points for a perfectly healthy bud patch, 4 for a lightly infected bud patch, 2 for a bud patch with lower half dead but with bud alive, and 0 for all others.

The other inoculation experiments were performed in May 1949; spores of *Diplodia* were used for inoculum. Under experiment A, ordinary green to green-brown budwood was used; and under experiment B, the extremes of green and of brown budwood were used to discover whether there was a difference in susceptibility between the two types of budwood. The spores were dusted over the budding after the bud patch was in place but before it was wrapped. Ten buddings were made for each treatment or factor tested. They were left wrapped for 20 days.

The period in which the buddings were made was relatively favorable, as indicated by the fact that the control series gave 80 percent success (table 2). Buddings inoculated with spores of *Diplodia* were all killed. Even buds given a previous treatment with Fermate were not protected against the infection provided by the method of inoculation used. This was to be expected, since the inoculum was applied around the cut edges of the budding, which were not covered with Fermate. The effect was the same on both brown- and green-barked budwood. Pycnidia of *Diplodia* were found in most of the dead bud patches.

TABLE 2.—*Effect on budding success of inoculation of top buddings by dusting with spores of Diplodia theobromae* [1]

Treatment	Budding success in—		
	Experiment A	Experiment B	
		Green budwood	Brown budwood
	Percent	*Percent*	*Percent*
Control	80		
Fermate-treated	100	100	100
Inoculated	0	0	0
Fermate-treated and then inoculated		0	0

[1] Percentage is based on 10 buddings.

EFFECT OF FUNGICIDES ON *DIPLODIA THEOBROMAE* AND HEVEA

To be suitable for use in budding, a fungicide should be toxic to *Diplodia theobromae* and nontoxic to the cambium of hevea. Budding tests showed that Fermate and, to a lesser extent, Arasan are satisfactory, and that others, such as Ceresan, Semesan, and sulfur, are unsatisfactory. Although the use of Fermate as a protective fungicide in budding operations gives satisfactory results, it has the disadvantage that its black color soils the hands and clothing of the budders. It was considered desirable, therefore, to find as good a fungicide that would not have this undesirable characteristic. Tests requiring actual budding operations proved laborious and were also wasteful of plant material; hence, a screening test was devised to determine the suitability of fungicides before trying them in actual budding. Fungicides used in the tests are listed in table 3.

TABLE 3.—*Fungicides used in the tests on Diplodia theobromae and hevea*

Fungicide	Active principle	Percent	Form
Arasan, Thiosan, Tersan, thiram.	Tetramethylthiuram disulfide	50	Powder.
Dithane D-14, nabam	Disodium ethylene bisdithiocarbamate.	25	Solution.
Fermate, ferbam	Ferric dimethyl dithiocarbamate	76	Powder.
Parzate, zineb	Zinc ethylene bisdithiocarbamate	65	Do.
Zerlate, ziram	Zinc dimethyl dithiocarbamate	76	Do.
Arathane WP-25	Dinitro capryl phenyl crotonate	25	Do.
Cuprocide	Red copper oxide		Do.
Elgetol	Sodium-dinitro-ortho-cresylate	19	Solution.
	Sodium butyl naphthalene sulfonate.	5	Do.
	Sodium chromate	2	Do.
G-4	Dihydroxy dichloro diphenyl methane.		Powder.
Isothan Q 15	Lauryl isoquinolinium bromide		Solution.
Mercuric chloride	Mercuric chloride	100	Powder.
Penicillin	Penicillin G, Na salt		Do.
P. E. P. S.	Polyethylene polysulfide		Suspension.
Phygon-XL	2, 3-dichloro-1, 4-naphthoquinone	50	Powder.
Santobrite	Sodium pentachlorphenate	85	Do.
Spergon	Tetrachlorobenzoquinone	98	Do.
Sulfur	Sulfur	100	Do.
Tennessee "34" copper	Copper basic sulfate	34	Do.
Vasco 4	Zinc oxide	72	Do.
	Zinc hydroxide	12	Do.
	Zinc sulfide	5	Do.

EFFECT OF FUNGICIDES ON *Diplodia theobromae*

To test the toxicity of the fungicides to *Diplodia theobromae*, glass rings were placed in petri dishes of potato-dextrose agar, measured amounts of the fungicidal mixture were placed within the rings, and the entire dish then was heavily dusted with *Diplodia* spores. The spores were obtained by inoculating 1-foot-long pieces of hevea budwood with *Diplodia*, which, when placed in a moist atmosphere, produced an abundant supply of spores in 5 to 10 days.

In all the tests made (table 4), Fermate was the standard control with which other fungicides were compared. The fungicides were divided into three groups on the basis of their effect on the growth of *Diplodia* in the petri dishes. These groups included: (1) Fungicides with no inhibitory effect; (2) fungicides that inhibited fungus growth only within the ring; and (3) fungicides that inhibited growth outside the ring.

Sulfur, Tennessee "34" copper, penicillin, Cuprocide, and P. E. P. S. (polyethylene polysulfide) did not inhibit fungus growth even at the highest concentration (table 4). *Diplodia* grew as well on the thick deposit of the fungicide within the ring as on the remainder of the dish.

The rest of the fungicides tested definitely inhibited the growth of *Diplodia*, especially at the highest concentration. Those in group 2, all of which are nonsoluble, were of approximately equal toxicity.

The fungicides of group 3, all of which are soluble, were in general more toxic than the nonsoluble ones. The basal concentration of mercuric chloride (a 0.2-percent solution) is equivalent in toxicity to the $\frac{1}{10}$ concentration of Puratized Agricultural Spray (a 0.005-percent solution of active principle); this indicates that under the conditions of the test the Puratized Agricultural Spray (phenyl mercury triethanolammonium lactate) is 40 times as toxic as mercuric chloride.

Table 4.—*Effect of fungicides at 3 different concentrations on the growth of Diplodia theobromae*

Fungicide	Basic concentration	Inhibitory effect[1] of concentration		
		Basic	1/10 basic	1/100 basic
Group 1 (No inhibitory effect):	*Percent*			
Sulfur	2	H	H	H
Tennessee "34" copper	2	H	H	H
Penicillin	(2)	H	H	H
Cuprocide	2	H	H	H
P. E. P. S	1	H	H	H
Group 2 (Inhibited fungus growth within the ring only):				
Fermate	2	N	L	M
Zerlate	2	N	L	M
Arasan	2	N	L	M
Parzate	2	N	L	M
Isothan Q 15	2	N	L	H
G-4	2	N	L	M
Group 3 (Inhibited fungus growth even outside the ring):				
Santobrite	2	14	4	N
Puratized Agricultural Spray	1	13	2	N
Dithane D-14	2	9	N	L
Mercuric chloride	.2	2	L	M

[1] Letters represent fungus growth within the ring: N, none; L, light; M, medium; and H, heavy. Figures represent zone width, in millimeters, of inhibition of fungus outside the ring.

[2] 100,000 Oxford units per cubic centimeter.

In the first three tests, Fermate did not inhibit fungus growth outside the ring, but in the fourth test marked inhibition beyond the ring was noticed at the highest concentration. This Fermate was from a more recent supply and differed from that previously used in that it had a grayish-black color, instead of being jet black, and in that it also was much easier to wet. Evidently it had a soluble component that was toxic to *Diplodia.* To test this further, another trial was made using: (1) Fermate as before, (2) Fermate washed five times with distilled water, and (3) filtered extract from the Fermate. All three proved to be equally toxic to *Diplodia.* The Fermate extract had a marked sulfurish odor.

Toxicity of Fungicides to the Cambium of Hevea

The toxicity of fungicides to the cambium of hevea was tested in conjunction with tests on the effect of the fungicides on *Diplodia theobromae*. These tests were made for the purpose of screening the fungicides used in budding trials. The tests consisted of a simulated budding procedure in which the fungicide was applied liberally to the hevea cambium as a dust or as a spray. A 0.5-percent-strength solution was used for fungicides in liquid form. The tests were qualitative in nature; the amount of fungicide applied to the cambium was much greater than the amount that would reach it during budding, when the fungicide is applied as a suspension or solution only to the outside surface of budwood and stock. Thus, if no toxicity were evident from the treatment, the fungicide could probably be safely used as a protectant in budding.

The reaction of the various fungicides is given in the following tabulation:

Fungicide:	*Reaction*
Fermate	Apparently nontoxic; cambium alive, with slight or no discoloration, and with flap still alive.
Zerlate	
Sulfur	
Arasan	
Parzate	
Dithane D-14	
Puratized Agricultural Spray	
Elgetol	
Spergon	Toxic; cambium dead, with brown discoloration, but with flap still green.
Vasco 4	
Arathane WP-25	
Phygon-XL	
G-4	Very toxic; both cambium and flap dead.
Isothan Q 15	
Santobrite	
Tennessee "34" copper	Very toxic; both flap and cambium dead, with discoloration extending 10 inches above and below the treated area in the form of streaks.

There was slight discoloration around cut edges in the control.

As could be expected from past budding results, Fermate and Arasan proved nontoxic to the hevea cambium. Parzate, Dithane D–14, Zerlate, Elgetol, and Puratized Agricultural Spray also were nontoxic to hevea. The first three were selected as sufficiently promising for actual budding trials. Elgetol and Puratized Agricultural Spray were ruled out, because of possible toxicity to the budders.

The simulated buddings in these trials were opened and examined after the relatively short period of 6 days. A further check on toxicity to the cambium can be made after 30 days to see if satisfactory regeneration of the bark has occurred. This was done with Fermate and Arasan, neither of which interfered with bark renewal.

FUNGICIDAL TREATMENT OF TOP BUDDINGS

Comparison of Various Fungicides

The fungicides chosen for experiments in treating top buddings were selected because they showed promise as protective agents in previous tests, being toxic to *Diplodia theobromae* and nontoxic to the cambium of hevea. The fungicides tested—Fermate, Zerlate, Arasan, Parzate, and Dithane D-14—were used at a concentration of 400 gm. per liter of water. This relatively high concentration of fungicide was used to insure that the concentration would not be a limiting factor in the test.

The top-budding clone used was F 409; the panel clone being top-budded was GA 1301, with branches of 1 inch to 1½ inches in diameter. The trees had not been sprayed for control of leaf blight, but they were in good condition. The budding was done in the latter part of December 1949. The trees were not yet suffering from drought, as the bark of both the budwood and the rootstock peeled readily.

The buddings were made without the use of a flap. Twenty buddings were made by each of 3 budders for each of the 6 treatments—a total of 360 buddings in all. New waxed-cloth tapes were used for wrapping. The buddings were unwrapped after 30 days.

Fermate, and to a lesser extent Arasan and Zerlate, gave markedly higher budding success than the control (table 5). Parzate and Dithane D-14 definitely gave better budding success than the control. In fact, there was a highly significant improvement in budding success, when analyzed statistically, where any of the fungicidal treatments were used. Fermate, Zerlate, and Arasan have the same methyl thiocarbamate grouping, and Parzate and Dithane D-14 have the same ethylene thiocarbamate grouping. Budding success seems to be correlated with the use of fungicides having such groupings. As both Parzate and Dithane D-14 are definitely toxic to *Diplodia*, the poor budding success attending their use as protectants may possibly be caused by the fact that they are toxic to or inhibit the growth of the cambium of hevea.

Table 5.—*Effect of fungicidal treatment of top buddings on percent*[1] *of budding success*

Treatment	Budding success			
	By budder No.—			Average
	1	2	3	
	Percent	*Percent*	*Percent*	*Percent*
Control	5	20	5	10.0
Fermate	100	85	80	88.3
Zerlate	90	70	70	76.7
Arasan	85	90	70	81.7
Parzate	30	30	45	35.0
Dithane D-14	20	30	45	31.7
Average of budder	55	54.2	52.5	----------

[1] Percentage is based on 20 buddings.

Many of the unsuccessful buddings treated showed pycnidia of *Diplodia* on the bud patches, indicating that infection took place from some area not covered by the fungicide. Attempts were made further to increase budding success by covering the buddings more thoroughly with the fungicide. In addition to giving them the regular treatment, all cut edges and exposed cambium of the budding were dusted with the fungicide after the bud patch was tied in place. This method was successful, but it proved too cumbersome to use on a commercial scale.

COMPARISON OF VARIOUS CONCENTRATIONS OF FERMATE

In preliminary tests Fermate gave satisfactory results in preventing fungus infection in buddings when used at concentrations of 200 to 500 gm. per liter of water. Three further tests were performed to determine the minimum concentration of Fermate that could be used and still obtain good budding success. Fermate was applied in the regular manner to the budwood and shoot. The buddings were wrapped with new tapes and were unwrapped after 30 days.

Three of the treatments—the control, Fermate at 100 gm. per liter of water, and Fermate at 200 gm. per liter—were repeated in all 3 tests (table 6). Each percentage figure in the table is based on 20 buddings, and 60 to 80 buddings were made for each treatment.

TABLE 6.—*Effect of concentration of Fermate on budding success with 3 commercial top-budding clones, during seasons relatively unfavorable for budding*[1]

Test	Budding material, date, and treatment	Budding success				
		By budder No.—				Average
		1	2	3	4	
A	Budding done March 10, 1950, using F 1620 on panel clone GA 1581:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
	Control	5	20	35	10	17. 5
	Fermate, 100 gm. per liter	100	75	85	100	90. 0
	Fermate, 200 gm. per liter	100	100	95	95	97. 5
B	Budding done March 8, 1950, using F 409 on panel clone GV 42:					
	Control	65	35	20	60	45. 0
	Fermate, 50 gm. per liter	100	90	85	95	92. 5
	Fermate, 100 gm. per liter	85	95	95	80	88. 8
	Fermate, 150 gm. per liter	95	90	100	90	93. 8
	Fermate, 200 gm. per liter	100	100	100	95	98. 8
	Fermate, 400 gm. per liter	100	90	100	100	97. 5
C	Budding done October 21, 1950, using FB 3363 on panel clone GV 42:					
	Control	4	8	4	------	5. 3
	Fermate, 100 gm. per liter	16	32	48	------	32. 0
	Control	32	0	4	------	12. 0
	Fermate, 200 gm. per liter	82	86	68	------	78. 7

[1] Percentage is based on 20 buddings.

The averages of the treated buddings that were successful under each test were higher than the average of the untreated buddings. In test B, even the lowest concentration of Fermate used, 50 gm. per liter of water, gave satisfactory results. In test C, however, Fermate at 100 gm. per liter gave an average success of only 32 percent compared with about 79 percent for Fermate at 200 gm. per liter. The higher concentrations of the Fermate, on the whole, gave better results than the lower. No detrimental effects resulted from the use of Fermate at the high concentration of 400 gm. per liter. It is concluded that a concentration of 200 gm. per liter of water is about the minimum amount that should be used for best results.

The type of budwood used, that is, whether it is green-barked and smooth or brown and corky, will influence the amount of Fermate that will adhere to the bark. Much more Fermate will adhere to rough, brown bark, and thus a lower concentration of Fermate can be used with this type of bark. However, it is much simpler to use an ample amount than to try to evaluate all the factors. Using a higher concentration will not increase budding costs appreciably.

Top Budding of Older Trees Using the Fermate Treatment

Sometimes it is necessary to make buddings on older trees with a diameter of three or more inches. This can be done by either of two methods. The tree can be pollarded to force new, young shoots that can then be budded, or it can be budded directly. Both methods have their advantages and disadvantages. Pollarding has the disadvantage that 6 to 12 months are lost, depending on seasonal conditions, in waiting for the new shoots to reach buddable size. Pruning has to be done in order to leave only three or four shoots. Sometimes the terminal buds of the new, succulent shoots are killed by fungus attack and spraying has to be done. Sometimes spraying is also necessary to control leaf blight. The main advantage of pollarding is that it is possible to bud new shoots in the regular manner.

Direct budding of the tree produces the desired top sooner. It has the disadvantage that budding is much more difficult and slower.

Very poor results were obtained in former years in Mexico in budding older trees, although satisfactory success had been obtained in Brazil and Costa Rica. Therefore, tests were made to determine if the use of Fermate would increase budding success and also to indicate whether *Diplodia* infection had been a major factor in the poor results obtained in budding larger trees in Mexico in past years.

Because of the thickness of the bark of old trees, a different budding technique had to be used. The budwood was of clone F 409, 1½ to 2 inches in diameter. Fermate, at a concentration of 300 gm. per liter of water, was applied in the regular manner to the budwood and to the tree after marking out the panel. In making the panels, which were about 1 inch wide and 3 inches long, a very thin-bladed knife was used to cut the thick bark of the trees. The bud patch was cut approximately the same size as the panel. The panel was removed, dipped in Fermate powder, and placed over the bud patch so that sufficient pressure would be applied to the patch on wrapping. Ordinary friction tape was used for wrapping, and the buddings were left wrapped for 25 days.

There was a marked improvement in budding success when Fermate was used (table 7). In the first test, all 10 untreated buddings were dead and 85 percent of 80 treated buddings were alive. In the second test, which had been preceded by several heavy rains, 30 percent of the 10 untreated buddings were alive as compared with 97 percent of the 30 treated buddings.

These results indicate that failure to use a protective fungicide accounted to a considerable degree for the poor budding success obtained in Mexico in past years in budding older trees.

TABLE 7.—*Effect of the Fermate treatment on top budding of large-diameter trees of standard panel clones*

Test	Date and treatment	Buddings	
		Made	Surviving
		Number	*Percent*
A	Buddings made on Sept. 12, 1949:		
	Control	10	0
	Fermate-treated	80	85
B	Buddings made on Oct. 20, 1949:		
	Control	10	30
	Fermate-treated	30	97

RESISTANCE OF BUDWOOD OF CLONES AND SEEDLINGS TO *DIPLODIA THEOBROMAE*

Better budding success can be obtained by using seedling rather than clonal budwood, and varying degrees of success may be obtained even between clones. For example, F 409 has at times given somewhat better success in being top-budded with resistant clones that have other clones such as F 1620, FB 3363, and FB 54. Likewise, better success generally has been obtained in budding seedlings (low budding) than in budding clones (top budding). Tests were made to determine whether there is any connection between budding success and resistance or susceptibility of budwood to *Diplodia theobromae.*

Seedling and various clonal budwood, 1 year to 1½ years of age and 30 cm. in length with no leaves attached, were inoculated on both ends by dusting with spores of *Diplodia.* For each type of material 6 to 11 sticks of budwood were used. They were wrapped in wax paper, and the amount of dead bark measured at 5, 10, and 15 days. Infection is easily observed, especially in green bark, by the blackening of the bark and by the fairly sharp demarcation between the infected and healthy tissue. It has been observed that green-barked budwood is more susceptible to infection than brown-barked and that the rate of advance of infection is almost invariably more rapid in the upper end of the stick than in the lower. The rate of advance is also influenced by the presence of buds. A corona of buds, for instance, has a tendency to slow up the advance. Initial infection of the budwood almost invariably occurs at the cut ends. In rare cases, it has started also from old leaf scars and, more commonly, in the cut petiole left after a leaf is trimmed off.

The rate of advance of infection is much slower in the seedling than in the clonal material (table 8). This phenomenon is clear-cut and has held for all seedlings tested in this and other trials. It is assumed that a reduced rate of spread in a stick of budwood is an indication that the budwood is resistant to *Diplodia.* Seedlings, therefore, apparently have some sort of inherent vigor or resistance that enables them to withstand attack by *Diplodia* better than the clones. This may partly explain why budding is more successful with seedling budwood than with clonal budwood. Nothing can be inferred from comparisons within the clones, as differences in infection between them at 10 or 15 days were not marked.

TABLE 8.—*Rate of advance of infection by Diplodia in budwood of seedlings and clones*

Plant material	Average dead bark per stick of budwood after—		
	5 days	10 days	15 days
	Centimeters	*Centimeters*	*Centimeters*
Seedlings:			
No. 1	4.7	10.5	25.2
No. 2	1.6	10.0	22.0
No. 3	3.8	14.2	22.6
Top clones:			
F 1620	9.5	30.0	----------
FB 3363	8.6	30.0	----------
FB 54	6.4	27.9	30.0
F 409	6.7	28.6	30.0
Panel clones:			
GV 37	4.8	30.0	----------
GX 26	4.6	29.9	30.0
GA 1301	4.5	26.2	30.0

Infection of budwood by *Diplodia* could be a very serious factor in shortening the life of budwood being shipped from one place to another. In a test, 10 sticks of budwood were treated with 20-percent Fermate and 10 others were left untreated as controls. All of the sticks were then liberally dusted with *Diplodia* spores and wrapped with wax paper to prevent desiccation. Within 10 days the control sticks were dead, but the Fermate-treated sticks were still alive after 20 days. This demonstrates the necessity of treating budwood before shipping, especially if there is to be a considerable lapse of time before it is used.

DISCUSSION

The Fermate treatment for the protection of buddings against fungus attack should be used whenever budding success falls below 85 percent. Poor results—caused by such factors as out-of-season budding, inferior budders, use of budwood not in first-class condition as in the case of undue delay in shipment, and budding of trees weakened by leaf blight—can usually be much improved[5] by proper use of the Fermate treatment.

Fermate should not be applied to the exposed cambium of the bud patch or shoot, although small accidental deposits do no harm. The treating of the budwood and shoots can be done by an unskilled laborer, and the budders can then finish their regular quota of buddings. One laborer is necessary for each five budders. A disadvantage of Fermate is that its black color soils the hands and clothing of the budders, but the Fermate can be removed with soap and water. A light cotton apron may be used by the budders to protect the clothing.

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[5] See footnote 4, p. 2.

In the preliminary trials the cloth tapes were also treated by dusting them with the fungicide, but this was found to be unnecessary with new tapes. However, it is considered best to dust with a fungicide tape that is to be used a second time.

The major factors that have a bearing on budding success in hevea are: (1) Vigor and activity of growth of the stock plant at and for a time subsequent to budding, (2) condition of the budwood, (3) use of correct budding procedure, and (4) attack by *Diplodia theobromae* or other fungi.

The best period for budding coincides with the most active period of growth of the plants. In Mexico, this period falls in June and July, about 1 month after the onset of the rainy season, and generally lasts for about 5 weeks. At this time the bark peels very easily and there is an abundance of sap in the cambial layer. Under such optimum conditions, 100-percent budding success can be obtained by good budders, even without the use of Fermate. However, there are periods in the year less optimum for growth in which the bark still peels readily but during which satisfactory budding success can be obtained only by the use of Fermate. By its use some measure of success can be obtained, even though the bark peels with difficulty and the cambium surface contains striations that can be seen and felt.

Budwood, the bark of which peels readily, can be used for budding purposes with every assurance of success. In general, it is better to select budwood of about the same diameter as the plants to be budded, as this allows the use of a wider bud patch and will give a better fit. If budwood is plentiful, smooth bud patches with minimum leaf scars should be selected, as the more even surfaces of such patches allow better contact of the two cambial layers and will, on the whole, give greater success in budding. The use of bud patches that have freshly cut petiole stubs will at times reduce budding success, for the reason that the stub is often attacked by *Diplodia* and the infection may occasionally spread to the bud patch. Unsuccessful buddings caused by this type of infection—the bud in the center dies but the rest of the patch remains alive—have frequently been found.

It is recommended that in the Northern Hemisphere buddings be made on the north or northeast side of the tree. This eliminates possible reduction in budding success as a result of overheating by the direct rays of the sun. Another effect of exposure to the sun in the case of Fermate-treated buddings is an increase in the length of time the buddings remain dormant after cutting back. No such effect has ever been noted when the treated buddings were made on a northern exposure.

One of the most important considerations in budding procedure is the tightness of wrapping. Within limits, the tighter a budding is wrapped, the greater is the likelihood of its success. Often the only difference between a good and a poor budder is the difference in the care and force they use in wrapping. The best budders occasionally break the 1-inch-wide cloth tapes used in wrapping.

The length of time a budding should be left wrapped depends on the rapidity of growth of the plant after budding. Under conditions favorable for rapid growth, the buddings can be unwrapped after 15 to 20 days, but they should be left wrapped for 30 to 35 days if growth is slow. The way to judge whether a budding has been left wrapped long enough is by the growth of the cambium around the bud patch. If the new cambial growth is pressing against the tape, the buddings can be safely unwrapped. Overgrowth above and below the tape is also an indication that the budding can be safely unwrapped. If the buddings are opened too soon, the thin layer of exposed cambium around the bud patch will dry up and be killed and at times even the bud patch will be affected adversely. The dead gaps of cambium around the bud patch of untreated buddings, occasionally found on opening them, are invariably caused by fungus infection, even though the bud patch itself is unaffected. Budding success is not lowered by leaving the buddings wrapped for excessive periods of time, although under such conditions the tapes become ruined and cannot be used again.

The common practice of nicking the bud patches on opening to see whether the budding has been successful is not recommended and should be avoided if at all possible. These fresh cuts expose the bud patch to possible drying or to attack by *Diplodia.* This is especially true when conditions are not favorable for the growth of the trees. If the budwood used in budding was green, the successful buddings can be easily identified by their green color. If a preliminary count is necessary at the time the bud is opened, it may be well to do it on a sampling basis. The final count can then be put off until the trees are cut back and the bud patches are more firmly established; this greatly reduces the possibility of damage through nicking.

Four clones (FB 3363, F 409, F 1620, and F 1619), all resistant to the South American leaf blight, were used to top-bud 20 high-yielding panel clones. More than 4,000 buddings were made in the most favorable period of the year. In this experiment, no difference was observed in the buddability of the above 4 resistant clones onto any of the 20 panel clones. In less favorable seasons, however, definite differences in buddability have been observed.

Several workers demonstrated that using budwood covered with heavy deposits of copper spray residues results in lower budding success (*5*). The tests on the toxicity of fungicides to the cambium of hevea showed that copper in the form of Tennessee "34" is toxic to the cambium. These tests agree, therefore, with the results obtained by those workers in the budding trials (*5*).

No experiments were conducted by the writer involving budwood covered with spray residues. However, in areas in which spraying with bordeaux mixture was done every 4 days, good top-budding success was obtained by using the Fermate treatment and a flapless budding panel. The use of Fermate and flapless budding panel apparently offsets any ill effects of the spray on top buds. Probably the poor low-budding success obtained with budwood covered with copper spray residues is due to the usual practice of making low buddings with a flap.

SUMMARY

Infection of the bud patch of hevea buddings by *Diplodia theobromae* was found to be a major cause of budding failure. When the budding was done during the periods unfavorable for the growth of the rubbertree, the infection at times also killed the cambium beneath the bud patch and even spread into the surrounding bark.

An effective and practical treatment was developed, using a protective fungicide that was toxic to *Diplodia* and nontoxic to the cambium of hevea. The fungicide, as a water suspension, is applied with a cloth to the budwood and around the budding panel of the stock plant prior to the budding operation.

A screening test was developed to determine the suitability of various fungicides for possible use as budding protectants. Of the fungicides tested, Fermate proved to be the most satisfactory. The lowest concentration of Fermate that gave consistently good results was a concentration of 200 gm. of Fermate per liter of water.

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